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# DEVELOPMENT OF A LIPID-ENRICHMENT TECHNIQUE FOR *ARTEMIA* JUVENILES PRODUCED IN AN INTENSIVE SYSTEM FOR USE IN MARINE LARVICULTURE

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## Introduction

Although there exists an obvious inclination to replace live larval food by formulated feeds, *Artemia* nauplii are still essential in the larviculture of marine fish species. So far, on-grown *Artemia* are rarely used in larviculture although they offer several advantages over *Artemia* nauplii: *e.g.* they contain a higher individual protein and energy content thus improving the fish larvae's energy budget; an equal amount of live food is reached with far less individuals thus a considerable saving of *Artemia* cysts; their composition of (n-3) highly unsaturated fatty acids (HUFA) may easily be improved by applying enrichment techniques (Lavens and Sorgeloos, 1991).
This study deals with the development of lipid-enrichment techniques adapted to the intensive culture of on-grown *Artemia*.

## Materials and methods

### Culture

Great Salt Lake (Utah, USA) *Artemia* were cultured for 7 days at 10 animals/ml on a dry feed (YM20). The water renewal was not renewed. Details will be published separately (Dhont *et al.*, in prep.).

### Enrichment

Seven-day-old *Artemia* juveniles were harvested, rinsed and transferred to a cylindro-conical tank at ±50 animals/ml. To the tank 0.6g.$l^{-1}$ HUFA enrichment emulsion (Selco) was added in one ratio (Léger *et al.*, 1987). This method was simplified by adding 0.6g.$l^{-1}$ Super Selco (SS) directly to the culture medium, skipping harvest and transfer. YM20, Selco, Super Selco, and Dry Selco are products of Artemia Systems SA, Gent, Belgium.

In later experiments, the three main aspects of this method were screened in search of an optimal enrichment procedure:

a. Enrichment product: SS (an emulsion containing ± 450mg HUFA.$g^{-1}$) *versus* Dry Selco (DS: a dry powder containing ± 150mg HUFA.$g^{-1}$);
b. distribution of enrichment product: classical (CLA) enrichment (Léger *et al.*, 1987) *versus* daily constant dose (DCD): every day an equal fraction of the total dose is added to the culture, *versus* daily increasing dose (DID): the first doses are smaller than in DCD but are increased daily as to reach the same total amount;
c. concentration of enrichment product: the standard concentration (0.6g.$l^{-1}$, as indicated in the guidelines of the enrichment products) *versus* higher concentrations; lower concentrations were applied in cases where water quality appeared to affect survival.

Low temperature storage

The administration of 6-day-old *Artemia* to cold-water fish larvae was simulated by transferring them to tanks filled with seawater at 12°C (halibut), 18°C (turbot) and 25°C (control). Survival and HUFA levels were monitored during 48h after transfer.

Enrichment of *Artemia* of various age and size

In fish larviculture it may be advantageous to offer gradually larger live prey. To cover prey sizes from nauplii to 7-day-old *Artemia*, the distribution of the enrichment medium was spread over 5, 4, 3, and 2 days, respectively, starting from day 0, but the total enrichment dose was kept at 0.6g.$l^{-1}$ by increasing the daily doses accordingly (Fig. 2A). All treatments were maintained for 5 days and analysed for HUFA content.

Results and discussion

Enrichment

The enrichment of *Artemia* juveniles with SS allows to build up similar HUFA levels as obtained with nauplii (Table I). These levels are, however, reached in a much shorter time span, thanks to the better developed filter-feeding apparatus of the juveniles. There was no difference in HUFA levels between juveniles that were harvested and rinsed prior to enrichment, and animals that were enriched directly in the culture tanks. The latter considerably simplifies the enrichment procedure.

HUFA levels showed considerable differences from one experiment to another (see standard errors in Table II). Apparently food uptake is strongly affected by water quality and animal condition. Gradual enrichment over a longer period (both DCD and DID) yielded much higher HUFA levels than the classical enrichment because *Artemia* could also accumulate lipids in its body tissue in addition to the lipids stuffed in its gut. DS and SS gave equal enrichment results but the failure rate was much higher (>60%) when using SS. The culture stability improved at 0.2g SS.$l^{-1}$ but at the expense of the HUFA level in the enriched *Artemia*. In all but one experiment, DID yielded higher levels than DCD. Final HUFA levels increased with increasing enrichment doses, at the highest dose the survival on day 7 dropped, however, to 30%. Based on these results 0.6g DS.$l^{-1}$ distributed as daily increasing doses was adopted as the standard enrichment procedure.

Table I. Comparison of enrichment yields in *Artemia* juveniles (own results) and nauplii (from Léger *et al.*, 1987)

| *Artemia* | Emulsion | Treatment before enrichment | Duration | 20:5n-3 | 22:6n-3 | Σ (n-3) HUFA |
|---|---|---|---|---|---|---|
| | | | (h) | (mg.g$^{-1}$ DW) | (mg.g$^{-1}$ DW) | (mg.g$^{-1}$ DW) |
| Nauplii | S | Harvest & rinsing | 12 | 7.9 | 4.4 | 14.4 |
| juveniles | S | Harvest & rinsing | 4 | 5.8 | 4.4 | 14.2 |
| | SS | Harvest & rinsing | 2 | 5.2 | 3.4 | 9.1 |
| | SS | None | 2 | 5.2 | 4.2 | 10.0 |

Table II. Enrichment results with different enrichment strategies, concentrations and products

| Product | Method | Conc. | 20:5n-3 | | 22:6n-3 | | Σ(n-3) HUFA | | Trials |
|---|---|---|---|---|---|---|---|---|---|
| | | g.l$^{-1}$ | mg.g$^{-1}$ DW | SE | mg.g$^{-1}$ DW | SE | mg.g$^{-1}$ DW | SE | |
| DS | CLA | 0.1 | 9.4 | 3.5 | 5.0 | 1.7 | 16.1 | 3.9 | 2 |
| | DCD | 0.3 | 10.0 | - | 1.4 | - | 11.7 | - | 1 |
| | | 0.6 | 34.9 | 15.8 | 11.8 | 7.5 | 49.7 | 24.8 | 11 |
| | | 1.8 | 38.5 | - | 17.1 | - | 59.5 | - | 1 |
| | DID | 0.6 | 44.2 | 13.5 | 16.5 | 7.0 | 64.3 | 21.3 | 8 |
| SS | CLA | 0.1 | 5.2 | 0.0 | 3.3 | 1.0 | 9.3 | 0.6 | 3 |
| | | 0.6 | 8.7 | 4.9 | 4.6 | 2.9 | 14.3 | 7.5 | 2 |
| | | 1.0 | 5.1 | - | 2.4 | - | 8.4 | - | 1 |
| | DCD | 0.2 | 9.4 | 2.2 | 1.1 | 0.6 | 10.8 | 3.0 | 8 |
| | | 0.6 | 22.8 | 5.9 | 6.7 | 0.7 | 31.3 | 6.9 | 2 |
| | DID | 0.2 | 12.1 | 1.8 | 2.1 | 0.9 | 14.7 | 2.8 | 8 |
| | | 0.6 | 36.0 | - | 14.0 | - | 53.0 | - | 1 |

Low temperature storage

HUFA levels dropped as the ambient temperature increased (Fig. 1). Apparently, starved *Artemia* thrive on their lipid reserves and metabolize less when the ambient temperature is lower. This implies that the feeding frequency is more critical in, *e.g.* turbot larviculture than halibut larviculture since preys that are not ingested within the first

hours after feeding will loose up to 25% of their HUFA. It is expected that cold storage of enriched *Artemia* juveniles (at 5°C) will allow to maintain initial HUFA levels at their maximum as was proven for nauplii for periods up to 48h by Léger *et al.* (1983).

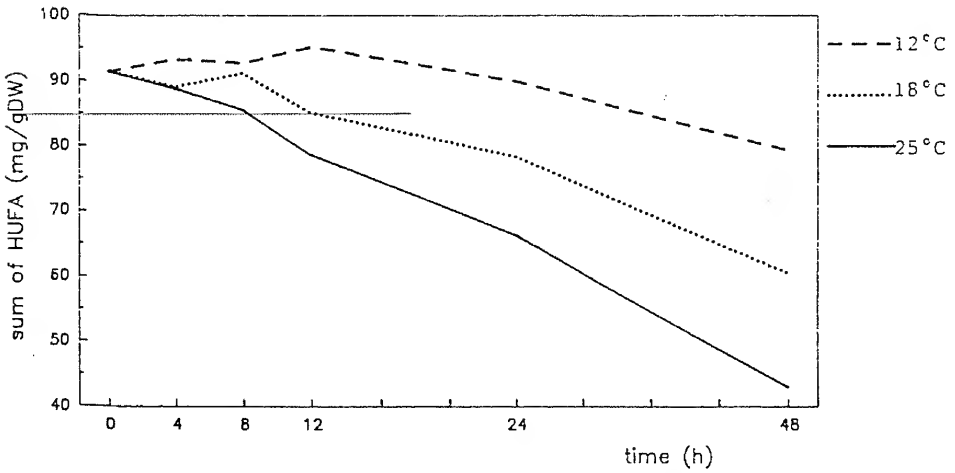


Fig. 1. Change in HUFA level of enriched *Artemia* stored at different temperatures.

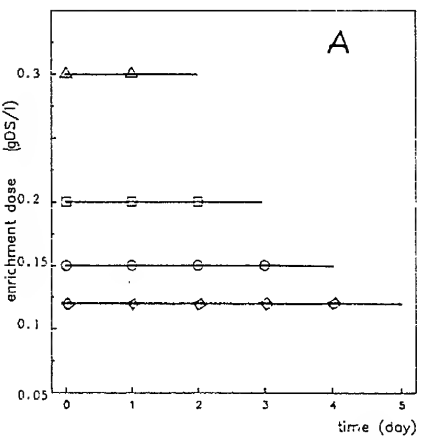


Fig. 2A. Enrichment doses of the different enrichment strategies; total enrichment dose is 0.6gDS/l for all treatments.

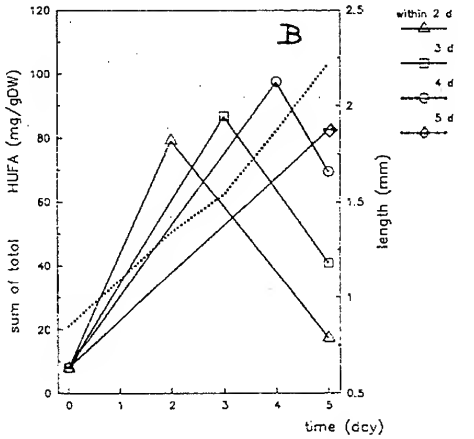


Fig. 2B. HUFA level in *Artemia* enriched within 2, 3, 4, and 5 days; the dotted line indicates animal size.

Enrichment of *Artemia* of various age and size

*Artemia* that received the total enrichment dose during days 0 to 2, accumulated HUFAs in their body tissue at levels comparable to those of gradually enriched 7-day-old *Artemia* (Fig. 2B). On the other hand, final HUFA levels still increased when the enrichment was spread over an extended period. Fig. 2 further shows that HUFA levels droppcd drastically as soon as the daily addition of the enrichment medium is ended. This implies that part of the assimilated HUFAs is directly metabolized. As a consequence, an increase in HUFA content will only be realized when the daily HUFA uptake exceeds its decrease through metabolism. This equilibrium determines the minimal effective daily enrichment dose, while its maximum is limited by its negative effect on water quality.

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